Megatrends in Bile Acid Receptor Research

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The Keystone Symposia conference organized by Luciano Adorini, Kristina Schoonjans, and Scott L. Friedman on "Bile acid receptors as signal integrators in liver and metabolism" in Monterey, California, March 3-7, 2017, offered the opportunity to assess state of the art research and delineate trends in this dynamic and very energetic field. The discovery of the nuclear hormone receptor superfamily was followed at the turn of the millennium by the breakthrough discovery that bile acids (BAs) are the endogenous agonists of the farnesoid X receptor (FXR) and subsequently also of the membrane G protein-coupled receptor TGR5. These seminal discoveries have sparked a dramatic transformation in BA research. In addition to understanding the role of BAs in regulating their own synthesis, metabolism, and transport, it is now clear that BA signaling in liver and intestine as well as in adipose tissue, kidneys, and muscles triggers signals required for the production, management, and storage of energy. In addition, the pleiotropic hormonal activities of BAs have been found to span from the regulation of lipid and glucose metabolism, to control of inflammation and fibrosis, to preservation of vessel integrity, to restoration of the intestinal barrier, to control of aging and circadian rhythms. Some of the dominant themes emerging from the stimulating discussions on the different facets of BA receptor (BAR) research among the over 250 delegates are outlined below and highlight the main trends of research in this young and exciting field.

Big Data and Systems Biology

The implementation of big data and systems biology approaches has clarified key facets of BAR biology, both in animal models and humans, and has accelerated translation toward novel therapies. Conceptually, systems biology approaches are especially vital to the study of BAR biology because crosstalk between the gut and liver as well as with adipose, muscle, and other tissues underlie many systemic effects of BAs and other nuclear receptor ligands. Moreover, these approaches may help dissect tissue-specific effects of BAs and their receptors, an aspect that is relevant because therapeutics are being developed that may target predominantly intestinal or liver receptors. The gut-specific FXR agonist fexaramine exemplifies ongoing efforts to use tissue-specific ligands to potentially avoid unwanted systemic effects.

In rodents, the use of multilayered "omics" strategies in model organisms has expanded our understanding of large networks of proteins and causal variants that may influence lifespan, blood pressure, and mitochondrial metabolism. For example, the BXD recombinant inbred mouse strain presented at the meeting has been established as a valuable genetic reference population to clarify the interactivity between energy metabolism and cellular signaling at the organismal level; it has emerged

Abbreviations: BA, bile acid; BAR, bile acid receptor; FGF21, fibroblast growth factor 21; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1; NASH, nonalcoholic steatohepatitis; OCA, obeticholic acid; TGR5, Takeda G-protein-coupled receptor 5.

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as a valuable platform for defining novel pharmacologic approaches to nonalcoholic steatohepatitis (NASH).

In humans, genome-wide association studies have uncovered variants that affect FXR/retinoid X receptor activation and regulate pathways of lipoprotein metabolism and inflammatory responses, among others. At the tissue level, global transcriptomic profiling of diseased liver has proven fruitful in defining dysregulated molecular pathways and molecular drivers of disease as well as defining targets for chemoprevention or treatment strategies that can attenuate the risk of cancer in chronic liver disease or treat the neoplasia that may complicate it. These comprehensive transcriptomic approaches are also used to optimize the development of suitable animal models to identify those with a gene expression pattern most closely resembling human disease.

Progress has not been limited to studies of gene expression and proteins but extends to the contribution of the epigenome and noncoding RNAs to disease causality and progression. For example, elucidation of the epigenetic regulation of hepatic stellate cell activation, the principle fibrogenic cell in the liver, has uncovered potential targets for antifibrotic therapy, including regulators of methylation and histone modifications. Similarly, long noncoding RNAs, about 200 nucleotide long, have been uncovered as modulators of cholestatic liver injury through their ability to regulate gene expression of effector molecules downstream of BAR signaling.

The liver is the focus of circadian regulation of metabolic pathways, especially those controlling the synthesis and metabolism of glucose, lipid, cholesterol, and BAs. Data presented at the meeting showed that circadian dysfunction in BA metabolism can promote NASH-induced hepatocarcinogenesis. *Fxr* is a clock-controlled gene and a chronic jet lag-type condition induces spontaneous hepatocellular carcinoma in wild-type mice following a mechanism very similar to that observed in obese humans. Studying the role of circadian homeostasis in controlling FXR function and response to FXR agonists will likely contribute to developing personalized BAR-based treatments in the future.

Membrane Signaling Pathways Downstream of BAs

Several novel molecular mechanisms mediated by BAs were revealed at this meeting. TGR5 regulates a plethora of nongenomic systemic metabolic actions of BAs and controls critical metabolic functions in a wide variety of tissues and cells, including liver, skeletal muscle, brown adipose tissue, and enteroendocrine L cells. TGR5 activation in the liver was reported to promote anti-inflammatory responses and to reduce portal pressure and steatosis. Another interesting observation was the finding that BAs can trigger glucagon-like peptide 1 (GLP-1) secretion from the basolateral compartment of enteroendocrine L cells, indicating that not only intestinal but also plasma BAs may contribute to the modulation of Ca^{2+} responses required for optimal GLP-1 secretion. Novel functions for TGR5 were also presented in other tissues. In the heart, TGR5 signaling favors glucose use, which is a source of energy under stress conditions and may protect against heart failure. BA pool modifications after vertical sleeve gastrectomy can decrease arterial blood pressure through a mechanism involving TGR5. Finally, TGR5 can coordinate the full program of beige adipocyte remodeling through mechanisms involving mitochondrial biogenesis and dynamics.

Other Emerging Membrane Signaling Pathways

Fibroblast growth factor 21 (FGF21) was a center-stage signaling molecule at the meeting. It functions as

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an endocrine hormone and is strongly induced in the liver under prolonged fasting. FGF21 signals through FGF receptors but requires β-Klotho as a coreceptor for efficient receptor engagement. Starvation increases hepatic expression of FGF21, which then targets mainly the central nervous system to increase hepatic gluconeogenesis, fatty acid oxidation, and ketogenesis. Pharmacological administration of recombinant FGF21 enhances insulin sensitivity, promotes body weight loss, and decreases triglyceride concentrations in mouse models. Although FGF21 is reportedly upregulated in fasting conditions, findings presented during the meeting revealed that the expression of FGF21 can also be modulated in the pancreas after feeding. Of interest, FGF21 has autocrine and paracrine effects in the pancreas, acting as a potent secretagogue to stimulate digestive enzyme release. In addition, FGF21 mitigates endoplasmic reticulum stress due to acinar cell protein overload.

Gut Microbiota and BA Interactions

The complex interactions among intestinal microbiota, BAs, host metabolism, and human health are currently the subject of intense research. The emerging view depicts the human body as a complex ecosystem of interacting prokaryotic and eukaryotic cells, with BAs playing a central role in the regulation of gut microbiota and reciprocally the gut microbiota shaping BA configuration. The BA pool composition is determined not only by primary BA synthesis in the liver but also by synthesis of secondary BAs by specific *Clostridium* species in the gut microbiome. *Clostridium scindens*, a BA 7α-dehydroxylating intestinal bacterium, is associated with resistance to *C. difficile* infection and, upon administration, enhances resistance to infection in a secondary BA-dependent fashion. Therefore, BAs can be viewed as signaling molecules linking the control of metabolic activities in the liver with the regulation of gene expression in specific gut bacteria that metabolize BAs. This liver–gut–BA axis becomes particularly relevant in liver disease.

A dedicated session at the meeting covered multiple aspects of the interplay between BAs and the gut microbiome, focusing on functional components of the gut microbiota–FXR–BA–liver axis. At present, a large fraction of studies in this field are carried out in mice, which are characterized by a BA composition that is very different from humans, and most of the results from these studies may not be directly translatable to human physiology. This is beginning to change, and at the meeting, the first data on the effect of obeticholic acid (OCA), a semisynthetic BA and a potent FXR agonist, on the human gut microbiome were presented. A shotgun metagenomic data set generated from fecal samples collected from healthy human subjects demonstrated a consistent increase in low abundance Gram-positive organisms in OCA-treated subjects, which returned to baseline after OCA was discontinued. This pattern was inversely correlated with plasma levels of C4, a BA precursor inhibited by OCA. In contrast, no association was observed for any of the BA-tolerant Gram-negative organisms with plasma levels of C4. Therefore, OCA induces a unique and reversible taxonomic signature in the human gut microbiome, indicating small intestinal bacterial BA sensitivity. These results provide the first glimpse of how the human gut microbiota may be modified by alterations in host BA synthesis. The analysis of the fecal microbiome, among other likely applications, highlights the potential of microbiota analysis for biomarker development to predict the response to FXR agonists in clinical settings.

Searching for FXR Agonists

While the search for the ideal FXR agonist as a therapeutic agent is actively ongoing, different views on the preferred features of FXR agonists to optimize their safety and efficacy were presented at the meeting. The BA derivative OCA, a steroidal semisynthetic FXR agonist, has shown clear clinical efficacy with a broad therapeutic window and manageable side effects. This has prompted the development of other BA-derived compounds specific for TGR5 or with dual FXR/TGR5 agonistic activity. All these semisynthetic BA derivatives behave like natural BAs in terms of metabolism and enterohepatic recirculation, with predictable pharmacokinetics and safety profiles in preclinical models. Conversely, nonsteroidal FXR agonists being currently developed typically possess higher potency in terms of FXR activation, usually display a systemic distribution, and are reportedly devoid of side effects considered related to BAs, for example, pruritus and modulation of the lipid profile. However, these may represent FXR-mediated class effects and are already being observed with some nonsteroidal FXR agonists. It remains to be seen how effectively and safely these nonsteroidal FXR agonists will translate into the clinic.

Different views were also presented at the meeting concerning the most suitable target organ for the treatment of liver diseases. Given the crucial role of the gut–liver axis and the expression of FXR in both organs, it has been argued that FXR activation in the gut alone would be sufficient to treat liver disease and perhaps be even safer and more effective than activating liver FXR. Consistent with this concept, gut-targeting FXR agonists could represent potentially interesting compounds, as noted above. However, BA-derived FXR agonists have already demonstrated that activating both liver and gut FXR can be clinically effective with manageable side effects.

Current Clinical Indications for FXR Agonists

The translational part of the meeting focused on proven clinical indications for FXR agonists: primary biliary cholangitis and NASH. The efficacy demonstrated by OCA in the treatment of primary biliary cirrhosis has established FXR as a valuable therapeutic target. In addition to the pivotal data from the PBC OCA International Study of Efficacy (POISE) trial in primary biliary cholangitis, ongoing phase 2 clinical studies testing OCA in primary sclerosing cholangitis and biliary atresia were also discussed.

However, the meeting spotlighted the potential of FXR agonists to treat NASH. Preclinical data demonstrate that FXR activation inhibits liver inflammation and fibrosis, promotes liver regeneration, controls intestinal bacterial growth, corrects dysbiosis, and is also involved at multiple steps in insulin signaling pathways. All these beneficial effects have been demonstrated in multiple models of NASH, using different FXR agonists. The meeting highlighted the rapid progress in the clinical translation of these findings, and several FXR agonists are currently in clinical development for the treatment of NASH, which is a major unmet medical need worldwide. For example, results were presented comparing the dual FXR/TGR5 agonist INT-767 to the GLP-1 receptor (GLP-1R) agonist liraglutide in mice with histologic features of NASH. INT-767 was shown to blunt these features in leptin-deficient ob/ob-NASH mice, as demonstrated by reduced fibrosis and decreased nonalcoholic fatty liver disease activity score. However, despite clear GLP-1R engagement by liraglutide, no histologic improvements were observed. In addition, in C57BL/6 mice with a high-fat diet-induced NASH, liraglutide improved only nonalcoholic fatty liver disease activity score features and steatosis while INT-767 prevented and reversed hepatic lipid accumulation, inflammation, and fibrosis through FXR-dependent mechanisms. These results suggest that FXR agonists do not require intact leptin signaling to improve NASH, whereas GLP-1R agonism may depend upon leptin to improve liver steatosis, with no apparent effect on other histologic features of NASH.

NASH is a complex disease with a multifactorial origin; therefore, it is likely that combination therapies will eventually be used. Among the various possible combinations, targeting the microRNA miR-21, which is greatly increased in the livers of NASH patients and in different animal models of NASH, coupled with OCA treatment was discussed. In preclinical models, this combination was found to inhibit NASH pathogenesis by modulating lipid metabolism and insulin signaling in hepatocytes and skeletal muscle as well as inhibiting hepatic inflammation and fibrosis. Once individual compounds are approved for NASH treatment, and even before, we will certainly see increased efforts toward combination strategies.

Future Clinical Indications for FXR and TGR5 Agonists

The meeting not only closely examined the potential of FXR agonists in the treatment of NASH but also explored a broad range of still untested clinical indications, using different preclinical models from cholestasis associated with total parenteral nutrition to prevention of hepatocarcinogenesis. Novel prospects for clinical translation of FXR agonists were exemplified by the efficacy of OCA in patients with BA diarrhea, highlighting the potential of FXR agonists beyond liver indications. The wide-ranging potential of FXR agonists indeed encompasses several indications in intestinal diseases (e.g., inflammatory bowel diseases) and in kidney diseases (e.g., diabetic nephropathy), but efficacy of FXR agonists has also been shown in preclinical models of lung diseases (e.g., pulmonary hypertension and pulmonary fibrosis). It can be anticipated that there will be a marked expansion of translational efforts testing FXR agonists in a wider range of clinical indications and likely also in organs that are not considered principal targets of its activity, for example, the lung or the brain.

Also, clinical indications for TGR5 agonists are expanding, based on preclinical work in rodent models. At the meeting, TGR5 was demonstrated to contribute

to arterial blood pressure lowering after vertical sleeve gastrectomy to beneficially modulate myocardial adaptation to stress and to induce beiging of white adipose tissue, highlighting its multiple positive effects linked to metabolic regulation. TGR5 remains an interesting target, but the side effects associated with its activation, including gallbladder enlargement and cardiac side effects observed with certain agonists, have considerably moderated the initial enthusiasm for clinical translation.

The Coming of Age of a New Field of Research

The success of the meeting was measured not only in the thorough assessment of the state of the art and in the brilliant results discussed but also in the recognition that a new field spawned by BAR research has emerged and is growing strong. Progress across a variety of interconnected aspects in this field is fostered by a vibrant multidisciplinary community with a variety of interests but a shared focus. The impetus thus far is expected to accelerate and will pave the way toward further progress, initially likely along the main trends we have briefly outlined here, but almost certainly in unanticipated directions as well.

Interpreting the interests of the scientific community and serving as a catalyst for the advancement of biomedical sciences, Keystone Symposia is already beginning to organize future liver-focused conferences that incorporate the latest BAR research. We are confident that soon there will be new developments to discuss and innovative advances to be disseminated.